ELSEVIER

Biochimica et Biophysica Acta 1268 (1995) 123–129

BBA Biochimica et Biophysica Acta

Mediation of pepsinogen secretion from guinea pig chief cells by Ca^{2+}/calmodulin-dependent protein kinase II

Naotsuka Okayama [a,*], Makoto Itoh [a], Takashi Joh [a], Tadahisa Miyamoto [a], Toshihiko Takeuchi [a], Tatsuo Suzuki [b], Akihiko Moriyama [c], Taiji Kato [c]

[a] *First Department of Internal Medicine, Nagoya City University Medical School, 1-Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467, Japan*
[b] *First Department of Biochemistry, Nagoya City University Medical School, 1-Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467, Japan*
[c] *Department of Bioregulation Research, Nagoya City University Medical School, 1-Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467, Japan*

Received 27 October 1994; revised 13 Febuari 1995; accepted 23 Febuari 1995

Abstract

In the presence of Ca^{2+} bound to calmodulin, Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II) exhibits an intramolecular autophosphorylation and modulates many cell functions. In this study, the role of CaMK II in pepsinogen secretion was investigated in cultured guinea pig chief cells by using a specific CaMK II inhibitor, 1-[*N*,*O*-Bis(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine (KN-62), and an antibody for the Thr-286-autophospholylated α subunit of CaMK II which specifically recognized the autophosphorylated form of CaMK II. KN-62 inhibited the pepsinogen secretion stimulated by carbamylcholine chloride, cholecystokinin octapeptide, and ionomycin in a dose-dependent manner without affecting intracellular Ca^{2+} concentrations, but had no effect on the secretion by 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) and forskolin. Heavy staining with the antibody for autophosphorylated CaMK II was observed in the cytoplasm of chief cells treated with carbamylcholine chloride or ionomycin, but only light staining was seen in cells treated with TPA or forskolin. Thus, CaMK II and its autophosphorylation may be a critical step in the intracellular pathway by which Ca^{2+} causes pepsinogen secretion from guinea pig chief cells.

Keywords: Pepsinogen secretion; Calcium/calmodulin-dependent protein kinase II; Monolayer culture; Calcium ion, intracellular; autophosphorylation

1. Introduction

Pepsinogen secretion is mediated by cAMP and Ca^{2+}/calmodulin-dependent signal transduction pathways in chief cells [1,2]. Both pathways require activation of specific protein kinases. CaMK II is a member of a family of Ca^{2+}/calmodulin-regulated protein kinases that includes myosin light-chain kinase, phosphorylase kinase, and Ca^{2+}/calmodulin-dependent protein kinases I and III [3–5]. Unlike the other kinases of this family, CaMK II can phosphorylate many proteins and modulate cellular events such as neurotransmitter exocytosis, catecholamine biosynthesis, cytoskeletal interactions, and glycogen metabolism [5,6]. The participation of this kinase in acid secretion was recently demonstrated in rabbit parietal cells [7–9] with the use of a specific inhibitor, KN-62 [10]. In addition, myosin light-chain kinase, a member of the same family as CaMK II, is involved in pepsinogen secretion [11]. These reports suggest that CaMK II may play a role in the secretion of pepsinogen.

In the presence of Ca^{2+}/calmodulin, CaMK II exhibits an intramolecular autophosphorylation that alters this enzyme to a partially Ca^{2+}/calmodulin-independent form [12–14]. The extent of CaMK II autophosphorylation can be assessed by an immunohistochemical technique with a specific antibody for the Thr-286-autophosphorylated α subunit of CaMK II [15].

In this study, the role of CaMK II in pepsinogen secretion was examined in cultured guinea pig chief cells by using both KN-62 and the antibody that recognizes autophosphorylation of this kinase.

Abbreviations: Carbachol, carbamylcholine chloride; CaMK II, Ca^{2+}/calmodulin-dependent protein kinase II; CCK-8, cholecystokinin octapeptide; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; KN-62, 1-[*N*,*O*-Bis(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine; KN-04, *N*-[1-[*N*-methyl-*p*-(5-isoquinolinesulfonyl)benzyl-2-(4-phenylpiperazine)ethyl]-5-isoquinolinesulfonamide; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate.

* Corresponding author. Fax: +81 52-8423316 or 8520952.

SSDI 0167-4889(95)00038-0

2. Materials and methods

2.1. Monolayer culture of guinea pig chief cells

Monolayer cultures of guinea pig chief cells were prepared as reported [11]. A male Hartley guinea pig (body weight 200–300 g) was anesthetized with ether, and the stomach was removed. After stripping the mucosal layer, the gastric mucosa was cut into small pieces (< 2 mm^2), incubated and shaken (160 oscillations/min) for 60 min at 37° C in Ca^{2+}-free medium [containing 24.5 mM Hepes (pH 7.4), 98 mM NaCl, 6 mM KCl, 2.5 mM sodium phosphate, 5 mM sodium pyruvate, 5 mM sodium fumarate, 5 mM sodium glutamate, 11.5 mM glucose, 2 mM glutamine, 0.1% (w/v) bovine serum albumin (fraction V; Seikagaku Kogyo, Tokyo, Japan), 0.1% (w/v) soybean trypsin inhibitor (Sigma, St. Louis, MO), 1% (v/v) essential amino acid solution (Whittaker Bioproducts, Walkersville, MD), and 1% (v/v) essential vitamin mixture (Whittaker)] with 0.1% (w/v) collagenase (Wako Pure Chemical Industries, Osaka, Japan). After passage through 200-μm nylon mesh, the dispersed cells were incubated and shaken for 30 min at 37° C in the same medium with 2 mM EGTA (Dojin Laboratories, Kumamoto, Japan). After passage through 80-μm nylon mesh, the cell suspension was centrifuged in Percoll solution (density, 1.129 g/ml) (Pharmacia LKB, Uppsala, Sweden) at $30000 \times g$ for 20 min. The chief cell-enriched fraction was collected by aspiration and suspended in culture medium comprising a 1:1 mixture of Ham's F-12 medium and DMEM (Gibco, Grand Island, NY) supplemented with 10% (v/v) fetal bovine serum (Whittaker), gentamicin (100 μg/ml) (Gibco), insulin (8 μg/ml) (Wako), and hydrocortisone (1 μg/ml) (Wako). The chief cell suspension was then planted into 12-well plates ($2 \cdot 10^6$ cells/well) coated with collagen type I (Sumitomo Bakelite, Akita, Japan) and cultured under 5% CO_2 in air at 37° C for 70 h (CO_2 incubator 6300; Napco Scientific, Tualatin, OR).

2.2. Pepsinogen secretion studies

The cell monolayers were washed three times with culture medium to remove floating cells before the addition of drugs to the culture medium and incubated under 5% CO_2 in air at 37° C for the appropriate time. Samples of culture medium were subsequently removed, and then pepsinogen secreted into the medium was measured by an enzyme immunoassay specific for guinea pig pepsinogen as previously described [11]. At the end of the experiment, the chief cells were removed from the plate by exposure to 0.04% (v/v) trypsin with 0.02% (w/v) EGTA, and counted. Pepsinogen secretion was expressed as nanograms of pepsinogen per $5 \cdot 10^5$ chief cells, and cell viability was determined by exclusion of trypan blue.

In preliminary dose–response studies, appropriate doses of carbachol (Sigma) and CCK-8 (sulfated form; Peptide Institute, Osaka, Japan), both of which stimulate the Ca^{2+} second messenger pathway; the Ca^{2+} ionophore ionomycin (Calbiochem, La Jolla, CA), TPA (Sigma), which directly stimulates protein kinase C; and forskolin (Wako) which stimulates the cAMP second messenger pathway, were determined. Carbachol ($n = 6$), CCK-8 ($n = 6$), ionomycin ($n = 6$), TPA ($n = 6$), and forskolin ($n = 6$) significantly increased the secretion of pepsinogen at concentrations of 10^{-5} M to 10^{-3} M, 10^{-6} M to $2 \cdot 10^{-5}$ M, 10^{-6} M to $2 \cdot 10^{-5}$ M, 10^{-8} M to 10^{-7} M, and 10^{-5} M to 10^{-4} M, respectively (Table 1). Concentrations that submaximally stimulate pepsinogen secretion (10^{-4} M for carbachol, 10^{-5} M for CCK-8, 10^{-6} M for ionomycin, 10^{-8} M for TPA, and 10^{-5} M for forskolin) were used in the following studies. Time course studies were performed to confirm the effects of these doses and to determine the appropriate incubation times. Because significant and submaximal increases in pepsinogen secretion were observed for all drugs 30 min after stimulation (Table 2), cells were subsequently incubated with each secretagogue for 30 min.

The effects of CaMK II on pepsinogen secretion stimulated by the secretagogues were determined by using KN-62 (Seikagaku Kogyo) and KN-04 (Seikagaku Kogyo), an inactive analog of KN-62 [16].

2.3. Determination of $[Ca^{2+}]_i$

For determination of $[Ca^{2+}]_i$, chief cells were cultured for 48 h on a collagen type I (Koken, Tokyo, Japan)-coated

Table 1
Dose–response relation of carbachol, CCK-8, ionomycin, TPA, and forskolin stimulation of pepsinogen secretion

Secretagogue	Secretagogue concentration (log M)									
	0	−10	−9	−8	−7	−6	−5	2× −5	−4	−3
Carbachol	159 ± 12	–	–	–	–	217 ± 52	453 ± 82 [a]	–	634 ± 121 [b]	728 ± 47 [b]
CCK-8	256 ± 71	–	–	–	339 ± 70	739 ± 112 [a]	847 ± 189 [b]	1220 ± 166 [b]	–	–
Ionomycin	354 ± 92	–	–	–	334 ± 29	2714 ± 187 [b]	3701 ± 294 [b]	4549 ± 542 [b]	–	–
TPA	94 ± 15	210 ± 39	1035 ± 85	4192 ± 511 [b]	5722 ± 636 [b]	–	–	–	–	–
Forskolin	146 ± 25	–	–	–	234 ± 18	435 ± 58	739 ± 48 [b]	–	873 ± 136 [b]	–

The chief cell monolayers were cultured at 37° C in the absence or presence of several concentrations of carbachol, CCK-8, ionomycin, TPA, and forskolin for 30 min. Pepsinogen secreted into the culture medium was measured by an enzyme immunoassay for guinea pig pepsinogen. Pepsinogen secretion was expressed as nanograms of pepsinogen (means ± S.E.M.) per $5 \cdot 10^5$ chief cells for 6 determinations.
[a] $P < 0.05$, [b] $P < 0.01$, compared to cells incubated without the secretagogue.

Table 2
Time course of basal and stimulated pepsinogen secretion by carbachol, CCK-8, ionomycin, TPA, and forskolin

Secretagogue	Pepsinogen secretion				
	0 min	30 min	60 min	90 min	120 min
Basal	120±10	230±38	227±46	371±80	591±91
Carbachol	89±11	734±121	764±112	813±94	967±137
CCK-8	106±9	702±80	884±133	953±175	976±133
Ionomycin	149±20	3559±269	3948±450	4306±363	4659±432
TPA	142±12	4192±511	5669±343	6523±628	6557±637
Forskolin	104±10	739±48	1080±79	763±64	1006±130

The chief cell monolayers were cultured at 37° C in the absence or presence of 10^{-4} M carbachol, 10^{-5} M CCK-8, 10^{-6} M ionomycin, 10^{-8} M TPA, and 10^{-5} M forskolin for 0–120 min. Pepsinogen secreted into the culture medium was measured by an enzyme immunoassay for guinea pig pepsinogen. Pepsinogen secretion was expressed as nanograms of pepsinogen (means ± S.E.M.) per $5 \cdot 10^5$ chief cells for 6 determinations, but basal secretion for 17 determinations.

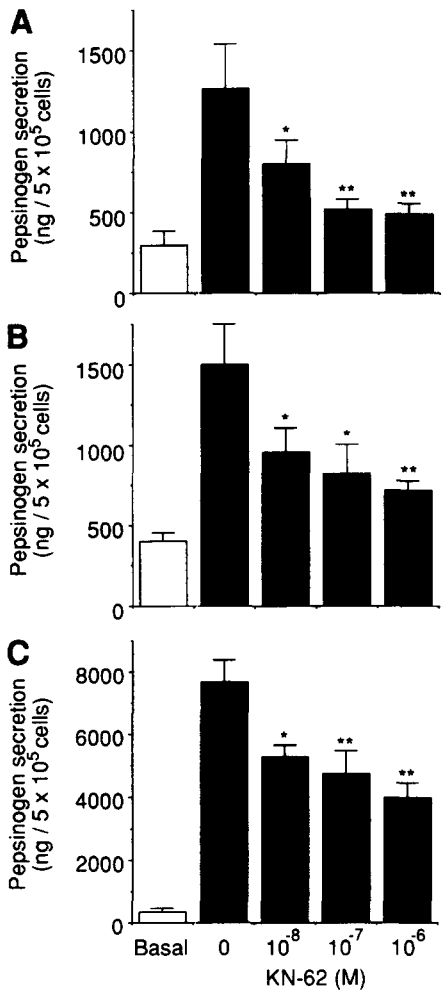

Fig. 1. Effects of KN-62 on pepsinogen secretion induced by carbachol (A), CCK-8 (B), and ionomycin (C). Cells were preincubated with KN-62 for 30 min before incubation with 10^{-4} M carbachol ($n = 6$), 10^{-5} M CCK-8 ($n = 6$), or 10^{-6} M ionomycin ($n = 5$) for 30 min (solid bars). Basal secretion is shown by open bars. Values are means ± S.E.M. * $P < 0.05$, ** $P < 0.01$, as compared to cells incubated with respective secretagogues but preincubated in the absence of KN-62.

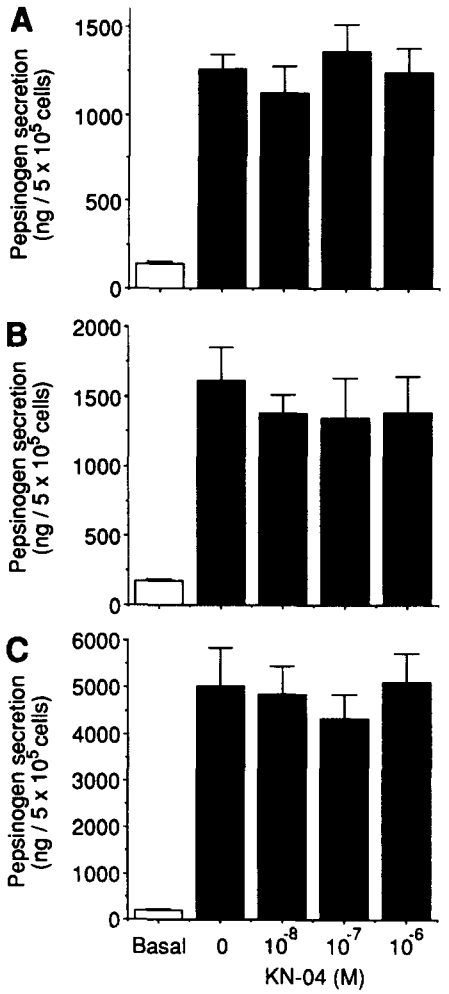

Fig. 2. Effects of KN-04 on pepsinogen secretion induced by carbachol (A), CCK-8 (B), and ionomycin (C). Cells were preincubated with KN-04 for 30 min before incubation with 10^{-4} M carbachol ($n = 5$), 10^{-5} M CCK-8 ($n = 5$), or 10^{-6} M ionomycin ($n = 5$) for 30 min (solid bars). Basal secretion is shown by open bars. Values are means ± S.E.M.

coverslip with a silicon rubber wall (Flexiperm-Disc; Heraeus Biotechnology, Hanau, Germany), after which the culture medium was replaced with a solution containing 130 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgCl_2$, 5.5 mM glucose, and 20 mM Hepes (pH 7.4). The cells were loaded with 5 μM fura-2 acetoxymethylester (Dojin Laboratories, Kumamoto, Japan) in the same solution for 60 min under 5% CO_2 in air at 37° C. The coverslip was mounted on an inverted epifluorescence microscope (model IMT-2; Olympus, Tokyo, Japan) with a xenon lamp and interference filters of 340 and 360 nm. Six cells were chosen at random in each preparation, and then illuminated by excitation beams of 340 and 360 nm, alternately (FC-200 type Ca^{2+} analyzer; Mitsubishi, Tokyo, Japan). Ratios of emitted fluorescence (fluorescence intensity under 340 nm divided by that under 360 nm) give the

absolute $[Ca^{2+}]_i$ after calibration. This system can monitor the changes in $[Ca^{2+}]_i$ in single cells [17–19].

The effects of KN-62 on the $[Ca^{2+}]_i$ induced by carbachol, CCK-8, and ionomycin were determined for 6 min in single chief cells.

2.4. Immunohistochemical studies

For immunohistochemistry, chief cells were cultured for 48 h on 4-well chamber slides (Nunc, Naperville, IL) coated with collagen type I. The cells were incubated in the absence or presence of carbachol, ionomycin, TPA, or forskolin for 30 min at 37° C in the culture medium, and then fixed overnight at 4° C with 4% (w/v) paraformaldehyde in phosphate-buffered saline containing 0.9 mM $CaCl_2$ and 0.5 mM $MgCl_2$. The cells were transferred sequentially to a permeative solution [0.1% (v/v) Triton X-100 in phosphate-buffered saline] for 15 min at room temperature, to primary antibody (IgG) for the Thr-286-autophosphorylated α subunit of CaMK II [1:100 dilution with phosphate-buffered saline containing 10% (v/v) fetal bovine serum] (given by T. Suzuki, [15]) or antibody preabsorbed with antigen (60 μg/ml) (peptide Y-66, which has the same sequence as the Thr-286-autophosphorylated α subunit of CaMK II [15]) (given by T. Suzuki, [15]) for 60 min at 4° C, and finally to secondary antibody [1:100 dilution with phosphate-buffered saline containing 10% (v/v) fetal bovine serum] (Cappel, Durham, NC) for 60 min at 4° C. The all immunohistochemical studies were replicated three times.

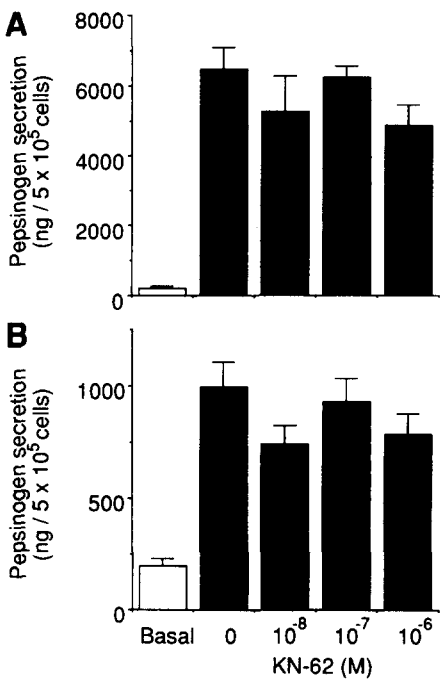

Fig. 3. Effects of KN-62 on pepsinogen secretion induced by TPA (A) and forskolin (B). Cells were preincubated with KN-62 for 30 min before incubation with 10^{-8} M TPA ($n = 5$) or 10^{-5} M forskolin ($n = 6$) for 30 min (solid bars). Basal secretion is shown by open bars. Values are means ± S.E.M.

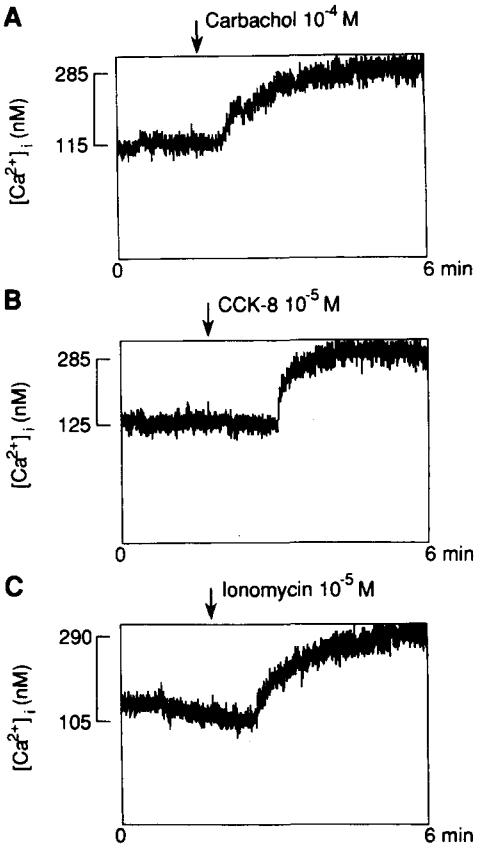

Fig. 4. Typical effects of KN-62 on the increase in $[Ca^{2+}]_i$ induced by 10^{-4} M carbachol (A), 10^{-5} M CCK-8 (B), and 10^{-5} M ionomycin (C). Cells were preincubated for 30 min with 10^{-6} M KN-62 for the experiments with carbachol and CCK-8, and with 10^{-5} M KN-62 for the ionomycin experiments.

2.5. Statistical analysis

Unless otherwise indicated, results are expressed as means ± S.E.M. Multiple comparisons of data from all experiments were performed with the Duncan test. Probability (P) values of < 0.05 were considered significant.

3. Results

3.1. Effects of KN-62 and KN-04 on pepsinogen secretion induced by secretagogues that stimulate the Ca^{2+} messenger pathway

Preincubation with KN-62 at 10^{-8}, 10^{-7}, and 10^{-6} M for 30 min significantly reduced the secretion induced by

10^{-4} M carbachol (36, 59, and 61% reduction), 10^{-5} M CCK-8 (37, 45, and 52% reduction), and 10^{-6} M ionomycin (31, 38, and 48% reduction) in a dose-dependent manner (Fig. 1). However, preincubation with the inactive analog of KN-62, KN-04, for 30 min did not affect pepsinogen secretion induced by the same secretagogues (Fig. 2). These effects of KN-04 confirmed the specificity of the inhibitory action of KN-62 on pepsinogen secretion induced by secretagogues that stimulate the Ca^{2+} second messenger pathway.

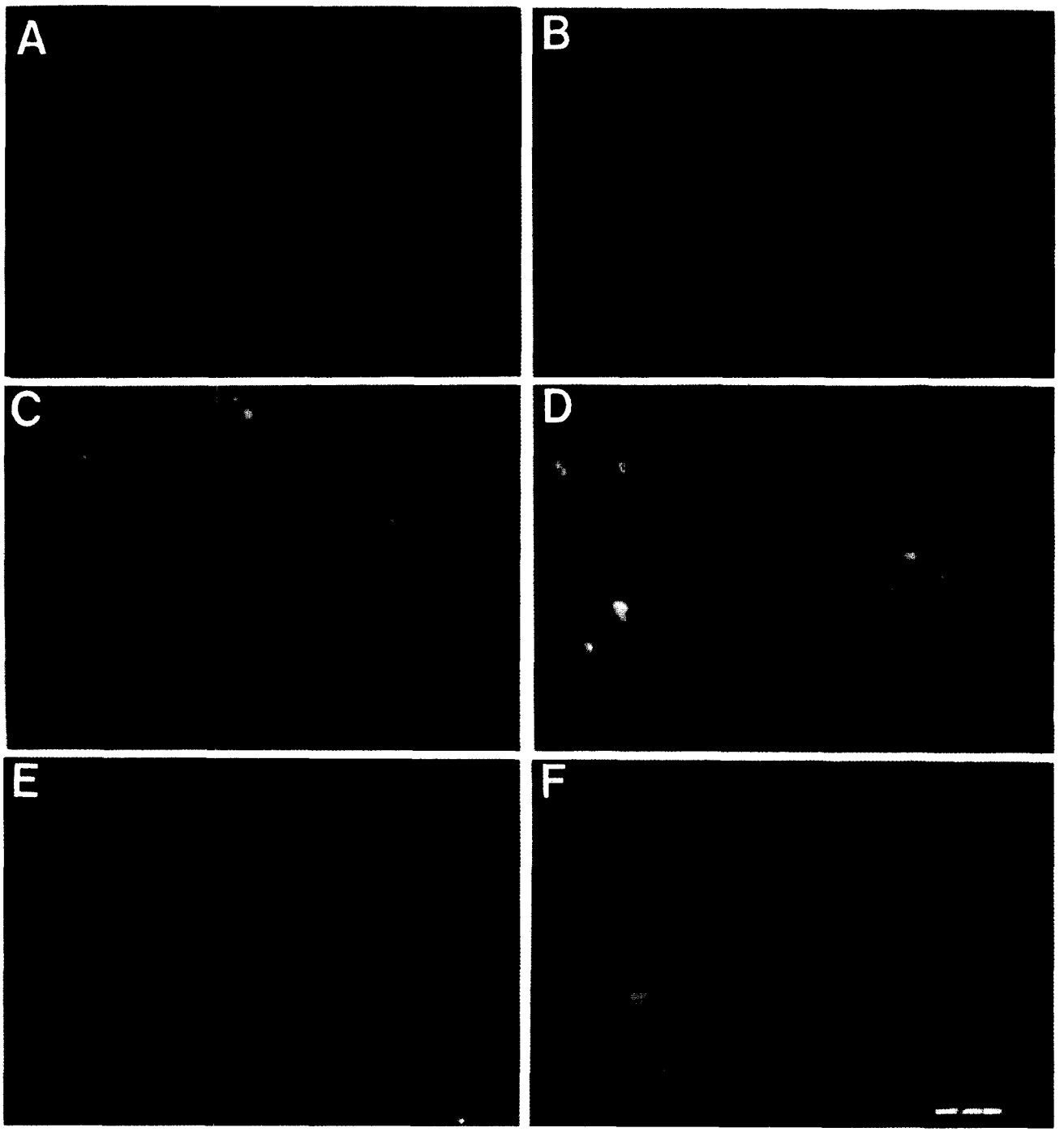

Fig. 5. Chief cells stained with antibody for the Thr-286-autophosphorylated a subunit of CaMK II without (B) or with 30 min stimulation by 10^{-6} M ionomycin (C), 10^{-4} M carbachol (D), 10^{-8} M TPA (E), and 10^{-5} M forskolin (F). As a negative control, the cells were stained with antibody that had been preabsorbed with peptide Y-66 antigen (A). The calibration bar in (F) indicates 10 μm.

3.2. Effects of KN-62 on pepsinogen secretion induced by secretagogues that stimulate protein kinase C or the cAMP messenger pathway

Preincubation with KN-62 (10^{-8}, 10^{-7}, and 10^{-6} M) for 30 min did not affect the secretion of pepsinogen induced by 10^{-8} M TPA or 10^{-5} M forskolin (Fig. 3).

3.3. Effects of KN-62 on the increase in $[Ca^{2+}]_i$ induced by carbachol, CCK-8, and ionomycin

A large increase in $[Ca^{2+}]_i$ was observed after stimulation of the cultured cells with 10^{-4} M carbachol, 10^{-5} M CCK-8, and 10^{-5} M ionomycin ($233 \pm 11\%$, $187 \pm 4\%$, and $230 \pm 19\%$ increase, respectively, from 6 cells). Ionomycin at a concentration of 10^{-6} M did not elevate $[Ca^{2+}]_i$; therefore, 10^{-5} M ionomycin was used in this study. Preincubation of the cells with KN-62 for 30 min did not affect the increase in $[Ca^{2+}]_i$ induced by the same concentrations of carbachol, CCK-8, and ionomycin ($231 \pm 23\%$, $197 \pm 17\%$ and $225 \pm 22\%$ increase, respectively, from 6 cells). The concentration of KN-62 was 10^{-6} M for stimulation with carbachol or CCK-8 and 10^{-5} M for that with ionomycin. Representative $[Ca^{2+}]_i$ traces revealing the effects of KN-62 are shown in Fig. 4.

3.4. Immunohistochemical studies on the autophosphorylation of CaMK II

The intramolecular autophosphorylation of CaMK II in chief cells was evaluated by immunohistochemistry with the antibody for the Thr-286-autophosphorylated α subunit of CaMK II (peptide Y-66). The cytoplasm and nucleus of untreated chief cells showed slight staining with the antibody (Fig. 5B), but did not stain with the antibody preabsorbed with peptide Y-66 antigen (Fig. 5A). Much stronger staining was observed in the cells treated with 10^{-6} M ionomycin (Fig. 5C) or 10^{-4} M carbachol (Fig. 5D), especially in the cytoplasm; however, only slight staining was observed in the cells treated with 10^{-8} M TPA (Fig. 5E) or 10^{-5} M forskolin (Fig. 5F). No staining with antibody that had been preabsorbed with peptide Y-66 was seen in the cells treated with the same concentrations of ionomycin, carbachol, TPA, or forskolin (data not shown).

3.5. Cell viability

Cell viability was determined by trypan blue exclusion after pepsinogen secretion studies. Viability of the cells that had not been exposed to drugs was $97.0 \pm 1.7\%$ (mean $\pm$ S.D.) for 33 separate wells. Cell damage, corresponding to a cell viability 2 S.D. below that of untreated cells, was not detected in any experiment (data not shown).

4. Discussion

CaMK II is distributed widely in many mammalian tissues [6,20–22] –the brain, islets of the pancreas, skeletal muscle, uriniferus tubules of the kidney, pituitary gland, and gastric glands [21]. Messenger RNA for CaMK II has been seen in the small intestine [22], indicating that CaMK II might also function in the alimentary tract. Recently, a specific inhibitor of CaMK II, KN-62, was synthesized [10]. KN-62 affects the interaction between Ca^{2+}/calmodulin complex and inactivated CaMK II by directly binding to the calmodulin binding site of CaMK II but does not affect the activity of already autophosphorylated (activated) CaMK II [10]. KN-62 inhibits more than 80% of the CaMK II activity at a concentration of 10^{-6} M; however, the activities of the cAMP-dependent protein kinase, myosin light-chain kinase, and protein kinase C, are affected only slightly in the presence of $10^{-4.5}$ M CaMK II (cAMP-dependent protein kinase, 22%; myosin light-chain kinase, 12%; protein kinase C, 12% of inhibitory rate) [10]. Because the inhibition of KN-62 against CaMK II was more significant than against the other protein kinase, KN-62 seems to be a selective and potent inhibitor of CaMK II. With the use of KN-62, an important role was established for CaMK II in acid secretion. KN-62 inhibits carbachol-induced acid secretion without alternating $[Ca^{2+}]_i$ in parietal cells [7]. However, the role of CaMK II has not been investigated in chief cells. We therefore tested the involvement of CaMK II in the secretion of pepsinogen from chief cells.

KN-62 specifically inhibited pepsinogen secretion induced by secretagogues that stimulate the Ca^{2+} second messenger pathway, such as carbachol, CCK-8, and ionomycin, in a dose-dependent manner. These results were confirmed by using KN-04; this inactive analog of KN-62 did not affect the secretion induced by such secretagogues, suggesting that the inhibitory effects of KN-62 on pepsinogen secretion did not seem to be non-specific. By contrast, KN-62 did not affect the pepsinogen secretion induced by TPA (a stimulator of protein kinase C) or forskolin (an activator of the cAMP second messenger pathway), neither of which affect $[Ca^{2+}]_i$ [23]. In addition, KN-62 did not affect the increase in $[Ca^{2+}]_i$ induced by carbachol, CCK-8, and ionomycin. KN-62 has been reported to inhibit insulin secretion from HIT-T15 cells by blocking Ca^{2+} channels of cell membrane because KN-62 had no effect on the secretion from permeabilized cells but inhibited the sustained rises of $[Ca^{2+}]_i$, which are due to Ca^{2+} influx via Ca^{2+} channels [24]. However, KN-62 did not affect the sustained rises of $[Ca^{2+}]_i$ induced by carbachol and CCK-8 in our studies (Fig. 4), impling that KN-62 may not act on Ca^{2+} channels of chief cell membrane. These findings indicate that CaMK II partly participates in Ca^{2+}-dependent secretion triggered by the elevation of $[Ca^{2+}]_i$ and is not involved in the secretion related to protein kinase C or

the cAMP second messenger pathway in chief cells. We previously reported that myosin light-chain kinase was also involved in pepsinogen secretion independent of protein kinase C and the cAMP second messenger pathway [11], indicating that the mechanism of activating CaMK II may be similar to that of myosin light-chain kinase in chief cells.

Triggering of intramolecular autophosphorylation of CaMK II by Ca^{2+} could prolong the effect of a transient increase in $[Ca^{2+}]_i$ and allow CaMK II to phosphorylate substrate proteins even when $[Ca^{2+}]_i$ returns to the basal concentration [5,6]. In this study, the autophosphorylation of CaMK II in chief cells was demonstrated by using antibody that specifically recognizes autophosphorylated CaMK II. The cytoplasm and nucleus of untreated chief cells contained small amounts of autophosphorylated CaMK II. However, much more autophosphorylated CaMK II was observed in ionomycin-or carbachol-treated cells compared to the cells treated with TPA or forskolin. These data suggest that the autophosphorylation of CaMK II is caused by the ionomycin-or carbachol-induced increase in $[Ca^{2+}]_i$, and that the autophosphorylated CaMK II probably stimulates pepsinogen secretion after $[Ca^{2+}]_i$ has returned to the basal concentration.

In conclusion, these studies imply that CaMK II and the autophosphorylation of this kinase may be involved in the Ca^{2+}-dependent pepsinogen secretion from chief cells.

Acknowledgements

We thank Dr. Kuniko Okumura-Noji, in our university, for preparation of the antibody for the Thr-286-autophosphorylated α subunit of CaMK II.

References

[1] Raufman, J.P. (1992) Gastroenterology 102, 699–710.
[2] Miyamoto, T., Itoh, M., Noguchi, Y. and Yokochi, K. (1992) Gut 23, 21–25.
[3] Nairn, A.C., Hemmings, H.C. Jr. and Greengard, P. (1985) Annu. Rev. Biochem. 54, 931–976.
[4] Nairn, A.C., Bhagat, B. and Palfrey, H.C. (1985) Proc. Natl. Acad. Sci. USA 82, 7939–7943.
[5] Schulman, H. and Lou, L.L. (1989) Trends. Biochem. Sci. 14, 62–66.
[6] Colbran, R.J., Schworer, C.M., Hashimoto, Y., Fong, Y.L., Rich, D.P., Smith, M.K. and Soderling, T.R. (1989) Biochem. J. 258, 313–325.
[7] Tsunoda, Y., Funasaka, M., Modlin, I.M., Hidaka, H., Fox, L.M. and Goldenring, J.R. (1992) Am. J. Physiol. 262 (Gastrointest. Liver Physiol. 25), G118–G122.
[8] Funasaka, M., Fox, L.M., Tang, L.H., Modlin, I.M. and Goldenring, J.R. (1992) Biochem. Int. 27, 1101–1109.
[9] Mamiya, N., Goldenring, J.R., Tsunoda, Y., Modlin, I.M., Yasui, K., Usuda, N., Ishikawa, T., Natsume, A. and Hidaka, H. (1993) Biochem. Biophys. Res. Commun. 195, 608–615.
[10] Tokumitsu, H., Chijiwa, T., Hagiwara, M., Mizutani, A., Terasawa, M. and Hidaka, H. (1990) J. Biol. Chem. 265, 4315–4320.
[11] Okayama, N., Joh, T., Miyamoto, T., Kato, T. and Itoh, M. (1994) Dig. Dis. Sci. 39, 2547–2557.
[12] Lai, Y., Nairn, A.C., Gorelick, F. and Greengard, P. (1987) Proc. Natl. Acad. Sci. USA 84, 5710–5714.
[13] Thiel, G., Czernik, A.J., Gorelick, F., Nairn, A.C. and Greengard, P. (1988) Proc. Natl. Acad. Sci. USA 85, 6337–6341.
[14] Schworer, C.M., Colbran, R.J., Keefer, J.R. and Soderling, T.R. (1988) J. Biol. Chem. 263, 13486–13489.
[15] Suzuki, T., Noji, K.O., Ogura, A., Kudo, Y. and Tanaka, R. (1992) Proc. Natl. Acad. Sci. USA 89, 109–113.
[16] Ishikawa, N., Hashiba, Y. and Hidaka, H. (1990) J. Pharmacol. Exp. Ther. 254, 598–602.
[17] Perney, T.M., Dinerstein, R.J. and Miller, R.J. (1984) Neurosci. Lett. 51, 165–170.
[18] Tsien, R.Y., Rink, T.J. and Poenie, M. (1985) Cell Calcium 6, 145–157.
[19] Kudo, Y. and Ogura, A. (1986) Br. J. Pharmacol. 89, 191–198.
[20] Hashimoto, Y. and Soderling, T.R. (1987) Arch. Biochem. Biophys. 252, 418–425.
[21] Fukunaga, K., Goto, S. and Miyamoto, E. (1988) J. Neurochem. 51, 1070–1078.
[22] Tobimatsu, T. and Fujisawa, H. (1989) J. Biol. Chem. 264, 17907–17912.
[23] Sakamoto, C., Matozaki, T., Nagao, M., Nishizaki, H. and Baba, S. (1987) Biochem. Biophys. Res. Commun. 142, 865–871.
[24] Li, G., Hidaka, H. and Wollheim, C.B. (1992) Mol. Pharmacol. 42, 489–498.